

CTL-derived exosomes enhance the activation of CTLs stimulated by low-affinity peptides

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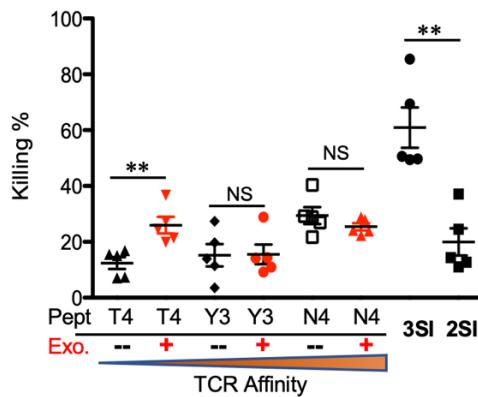
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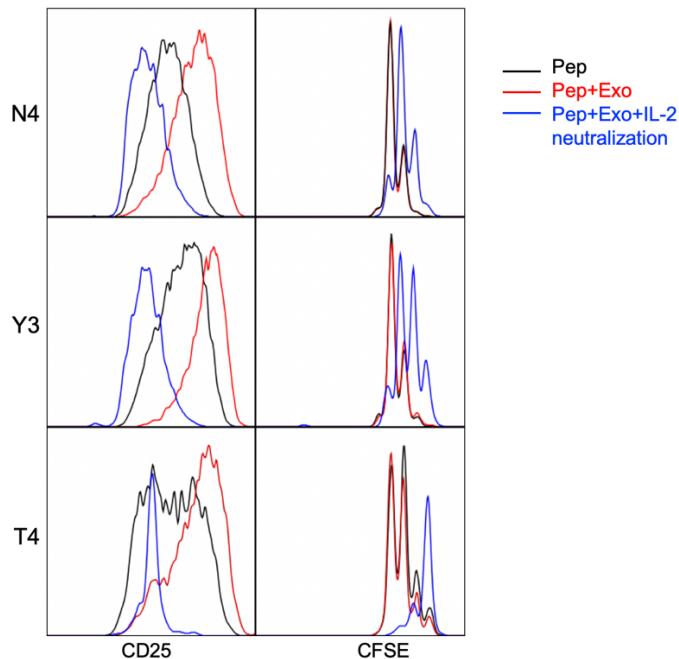
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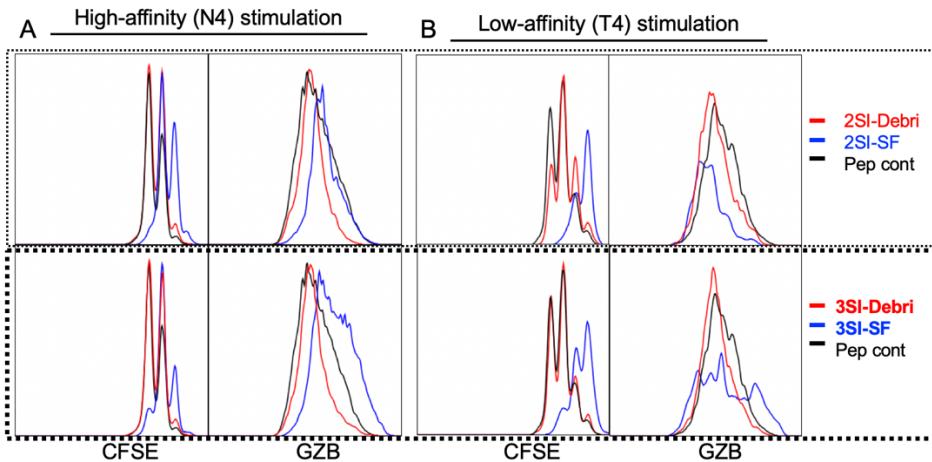
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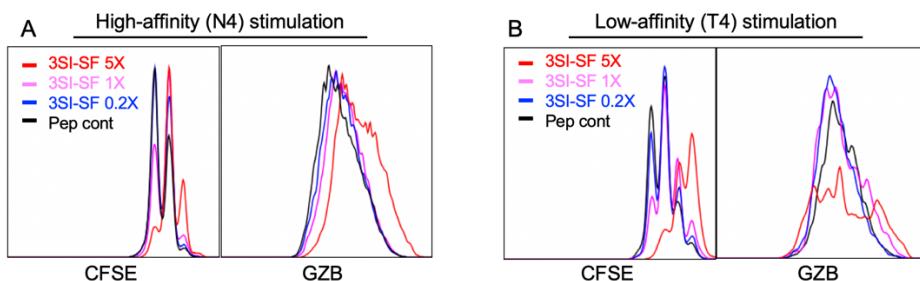
Supplementary Fig 1. Exo enhances the killing ability of low-affinity stimulated CTLs. Naive OT-I cells were stimulated with peptide with or without Exo, before seeded with B16.OVA at the ratio of 10 (effector) : 1 (target). Killed % = 100% x (RLU of untreated B16.OVA cells – RLU of B16.OVA cells cultured with OT-I cells)/RLU of untreated B16.OVA. Data are representatives of 3 experiments.



Supplementary Fig. 2. IL-2 is required for the effects of Exo. Naive OT-I cells were labeled with CFSE and stimulated with peptide with or without Exo or (Exo+IL-2 neutralizing antibody) for two days. Exo: exosomes secreted by 3SI-stimulated CTLs. Data are representatives of at least 3 experiments.



Supplementary Figure 3. Cell debris does not enhance the activation of CTLs stimulated by high- or low-affinity. Naive OT-I cells were cultured with dimer X coated plates pulsed by either N4 (A) or T4 (B) peptides in the presence or absence of cell debris at a ratio of 20 to 1 (debris from 20 activated CTLs was added to one stimulated CTL). Soluble fractions (SF) were similarly examined, at a protein concentration 5-fold of exosomes, which is $5 \times 33 \mu\text{g/mL} = 165 \mu\text{g/mL}$ in A-B, or at 165, 33, and 6.6 $\mu\text{g/mL}$ in E. CTLs were harvested for staining two days after culture.



Supplementary Figure 4. Effects of soluble fractions (SF) are dose-dependent. Naive OT-I cells were cultured with dimer X coated plates pulsed by either N4 (A) or T4 (B) peptides in the presence or absence of soluble fractions (SF) at a protein concentration 5-fold (5X), 1-fold (1X) or 0.2-fold (0.2X) of exosomes, 165, 33, and 6.6 $\mu\text{g/mL}$ respectively. CTLs were harvested for staining two days after culture. Data in Suppl. 3 and Suppl. 4 were generated in the same experiment, but presented separately for the purpose of easy comprehension.

Supplementary Table 1. Total proteins detected in D1E and D3E. Three different biological replicates of D1E and D3E were analyzed by proteomic assay. The detection of protein was only validated if its presence occurred in all three replicated. The list of proteins contains all proteins identified in either D1E, D3E, or both.

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Supplementary Table 2. D1E differential proteins. This list includes proteins either only detected in D1E, or, at least 1.5-fold higher in abundance in D1E than in D3E, as calculated by a comparison of the average abundance of each detected protein in reference to GAPDH, the internal control.

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P19788
E9Q3X0
B7Z9C2
E9PW66
P13595
Q8K1X4
Q9QYG0-2
P14543
P10493
E9QN31
Q9D6Z1
E9Q7G0
Q8QZR4
Q8WWZ8
Q9NRN5
O35103
Q9HBI1
Q9NPG4
Q9UN70
Q15113
P30086
Q5EBQ2
P08567
Q9DC11
Q62009
Q92954
Q99PV0
B1AT82
Q3TXS7
Q8VDM4
Q61838
P02753
P26043
Q9HD89
Q91VI7
P47962
Q5EBQ6
P62281
Q5M9L7
Q01730
P80511
Q3TGX0
A0A0U1RQQ9
E9Q1G8
E9QLL8
P50454
P19324
Q921M3
Q8VIJ6
D6W5L6

A0A0R4J2D8
Q62376
Q8K1I3
P56873
P50502
P54116
B2RUC7
A2AVA0
P37802
Q9WVA4
O08710
Q15582
P07996
P35443
B2RTL6
P39876
Q9Y490
P26039
P24821
A0A0B4J2C3
P01849
P68369
P68373
A0A0A0MQA5
Q9D6F9
P68372
Q8K0T7
Q6EMK4
P50552
P70460
P20152
E9QPU1
O75083
O88342
Q6ZQL4
Q5SS40
A8IP69
F6VW30
P09470
Q76LX8
Q769J6
Q8CG16
P01024
A0A0G2JPR0
P07384
K7ERG9
Q9D1A2
Q9Y6Z7
P53634
Q9WUU7
P00742
P00740
Q9Z0L8
P08882

Q14520
P08071
P98064-2
P48740
O00187
P14780
P30101
P27773
A2RS23
Q9QUR6
Q792Z1
I3L0U2
Q9R1P4
P49722
Q58EV4
Q9R1P0
Q9QUM9
O14818
Q9R1P1
P99026
Q60692
Q60841
Q9QXD6
P70695
Q8N0Y7
P62141
P63330
Q64487-10
A2A8L5
Q13332
Q7TPR4
P14211
Q6ZQ38
P27661
Q8BV49
Q96JK9
Q921D4
Q9R190
P58546
Q3U2W2
P97798
Q61982
O70400
P62196
Q60973
P11031
Q62318
P62960
P31946
A0A087WQS2
Q9D8N0
Q3ULL5
Q8R1B4
Q9QZD9

Q6NZJ6
F5BFH0
Q9UGM3
P11717
Q07113
Q61730-3
P40189
Q9QUM0
P09055
P05106
A0A0R4J0I9
Q64449
Q9UBG0
O14786
P97333
Q6PDS3-3
P15702
P19320
P01023
Q546G4
P08758
E9Q414
P04114
B0YIW2
O95445
Q9Z1R3
F6Q8D3
O55143
Q9CYN9
Q9JIF7
O55029
Q16610
P21995
Q8BG05
Q8VEK3
Q9DB05
P61971
Q8CG48
Q8C788
A0A0R4J0R1

Supplementary Table 3. D3E differential proteins. This list includes proteins either only detected in D3E, or, at least 1.5-fold higher in abundance in D3E than in D1E, as calculated by a comparison of the average abundance of each detected protein in reference to GAPDH, the internal control.

ID

P01580
P34884
P26883
P32883-2
Q9WTI17
Q4FJL0
P63321
P97855
P97310
Q9JJ00
Q9JIZ9
P61226
Q9CQI3
P67871
P11440
P43404
P70372
Q5M9L0
Q61699
Q5M9M0
Q5I0T8
P41105
P62889
A0A0A6YX26
P62911
P62841
Q9CZX8
Q5BLK2
P62852
P62855
A0A0R4J0T5
Q9JI48
Q3UEB3
P35979
Q4VAG4
Q6ZWZ4
Q58E35
Q9D8S5
P62320
P63163
Q99N09
D3Z2H9

O54901
P18181
Q3TEK8
Q6PFA2
Q64735
P05533
Q01965
Q3TA56
P61290
Q91Y47
Q02105
P70670
O70251
Q6ZWX6
Q9DCH4
P62267
P22646
B1B507
Q9QUM4
Q9D1C8
Q52L97
P97370
P53986
Q3UDP9
Q09143
Q9Z127
P01590
Q3TZH4
Q3UIG0
P18572
Q5SQB7
P32037
P08030
P13597
Q9CYL5
P47963
P01887
P01831
Q6ZWV3
P63213
P10852-2
P14206
Q58EW0
P35762
P62918

Q549Q4
Q99KN1
H7BX95
Q58E49
Q8C708
A0A0R4J1E2
P12970
S4R1S4
Q542I2
Q564E8
P35700
Q01853
Q4FK49
Q543H0
P62835
E9QB02
P08249
Q3TQ70
P68040
Q922B2
P62071
Q99JI4
Q00612
P08752
Q9QUJ7
Q99KP6-2
Q3TQX5
Q9DCL9
B2RQC6
Q3V3E1
Q5YLW3
O08992
Q01320
Q9JIF0
Q3UL22
Q9DC51
P19157
P84084
Q8BGQ7
P17918
P11499
Q922D8
Q1HFZ0
Q8CGC7
Q790I0

Q8C266
F8WIT2
Q9Z1Q5
A0A0A6YWM8
Q99KH8
E9Q696
O55098
O70293-2
P25444
Q5M9M4
Q9CVB6
Q9JLZ6
Q3UKW2
Q8BP67
P62849
P80315
Q4KML7
Q9CR57
P61255
Q5SUA5
A2APM2
Q9Z123
Q92747
A0A0R4J117
Q8BL97
P34022
Q545X8
P70441
P80317
Q60864
P27659
P63037
Q91V55
P99027
Q6ZWZ7
P80313
Q5XJF6
P59999
Q8QZY1
Q5SXR6
Q9ES61
Q9D1R9
Q497E9
Q9WU78
Q99JX4

P62830
Q3UN51
Q3U2G2
P97351
P14115
Q8R0J7
P21956
Q9JM76
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P11983
E9PYL9
P18760
P07356
P01899
P12815
Q54AJ5
P26041
P14131
P97371
P01901
Q9WVK4
O09167
P61161
Q92820
Q64514-2
Q8BVQ9
P70677
P28843
Q61187
P14148
Q9WTM5
Q921F2
P50580
Q9Z0N1
Q6ZWY3
Q8R050
P62264
P29341
Q61735-2
D3Z627
Q3UP78
P31041
A2AW86
Q61790
O35714

P11835
Q61003
Q62351
Q9ESU7
Q91YH6
P17809
Q9QZE5
Q8VDN2
Q3U0D7
P61923
Q8BY89-2